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Some properties of the virus extract of dock mosaic. By JOHN GRAINGER, B.Sc., and GEORGE COCKERHAM, B.Sc. (Communicated by Professor PRIESTLEY).

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The appearance of an Infectious Chlorosis on the Dock (*Rumex obtusifolius* L.) was noticed by the first mentioned author in November 1928.[1] The object of this paper is to give an account of experiments conducted during the summer of 1929, in order to determine something of the nature of the disease. With this end in view, the most natural line of attack appeared to be that of determining, under various conditions, the behaviour of the extracted sap of diseased plants.

A hundred seedling docks were obtained from Slaithwaite, near Huddersfield, where the chlorosis was not widespread, and were transplanted to 4 in. pots. One whole compartment of an insect-proof greenhouse was reserved for their growth. A few days after the introduction of the docks into the house, they were found to be covered with aphids. The compartment had been previously free from such insects and as the seedlings had been examined when planted, it was concluded that the aphids had been carried in with the docks, but had been hidden from observation. Quassia wash applied directly to the plants quickly removed most of the insects and a heavy fumigation with nicotine shreds completed the process. All the plants were then healthy, and remained so until needed for experiment.

All inoculations were made in the greenhouse and the plants remained there until a period of very warm weather in July caused fears—later proved justifiable—that symptons would be masked by the high temperatures obtaining in the greenhouse at this time. The plants, therefore, were removed to a border of sand surrounding the buildings, and protected by wire netting from chance depredations. Under these conditions the docks remained reasonably free from insect attack and chance infection as proved by the continued health of the twenty control plants.

The extracts used throughout the following experiments were prepared by finely grinding, in a mincing machine, leaves from infected docks. The resulting pulp was squeezed through fine muslin, when a green-coloured liquid—the "virus extract"—was obtained. Inoculation was carried out by wetting the fingers with the virus extract and then rubbing them on the leaves of healthy plants so that holes were made in the latter. The fingers were dipped very frequently in the inoculum and were well washed between the experiments.

The effect of dilution of the extract

A dilution of the original juice of 1 in 10 was made by thoroughly mixing 1 cc. of the extract with 9 cc. of distilled water. By taking 1 cc. of this dilution and adding 9 cc. of distilled water a dilution of 1 in 100 was obtained and a further dilution of 1 in 1,000 was prepared similarly. Four plants were inoculated from each preparation.

Results

Dilution of inoculation	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$
No. of plants inoculated	4	4	4
No. of plants diseased	3	2	0

The extract was, therefore, infectious at a dilution of 1 in 100, but not at 1 in 1,000.

Filtration experiments

Filtration through porcelain blocks which do not allow bacteria to pass is regarded as a criterion of the virus nature of an infectious chlorosis. The virus extract of dock mosaic was drawn through a clarifying filter of paper pulp and sand and the filtrate passed through a Jenkins' filter under the suction of a water pump. Tissue-mutilation inoculation with the ultimate filtrate on to five healthy dock plants induced no disease in the latter.

Some of the clarified juice was passed through an English Berkefeld candle, the resulting filtrate being inoculated on to twelve healthy docks, none of which showed mosaic symptoms.

This failure of the filtrates to infect must be considered in relation to the experimental results of the work on dilution. It is known that the process of filtering the extract of tobacco mosaic usually results in its dilution to 1 in 100 or 1 in 1,000 of the original strength. It is reasonable to suppose that the same order of weakening occurs when dock mosaic extract is filtered, and since this liquid is inactivated by dilution to 1 in 1,000, it is highly probable that the process of filtration inactivates the extract by the mere effect of dilution.

Effect of age upon the extract

This was studied by allowing the expressed juice to stand in a small corked bottle on the laboratory table and at intervals inoculating healthy dock plants with the extract. It was found that two plants became diseased out of five inoculated at the end of 14 days and disease was always produced by inoculations at shorter intervals.

Diseased leaves were allowed to dry in the air and inoculum was made from them by grinding in a mortar with a little water. After 21 days, out of five plants inoculated two produced typical mosaic symptoms. These experiments were not extended beyond the 14 days in the case of the extract, and 21 days in the case of the dried leaves.

The effect of alcohol and formalin on the virus extract

The effect of these two chemicals was studied by using the expressed sap of diseased plants in place of water for the preparation of the concentration indicated, e.g. 70% alcohol was prepared by adding 7 cc. of absolute alcohol to 3 cc. of the virus extract. The mixtures were inoculated into healthy docks by leaf mutilation half an hour after their preparation.

Results

Strength of chemical used	No. of plants inoculated	No. of plants diseased
50% Alcohol ...	5 ...	3
70% ,, ...	5 ...	3
95% ,, ...	5 ...	2
2% Formalin ...	5 ...	0

The virus extract of dock mosaic is, therefore, not inactivated by treatment with 95% alcohol for half an hour, but is destroyed by 2% formalin for the same time.

The effect of heat on the virus extract

This was studied by placing about 3 cc. of the extract of diseased plants in a thin-walled test tube and half-immersing the latter in a bath of water at the required temperature. This temperature was maintained for ten minutes, after which time the liquid was cooled rapidly and used to inoculate healthy plants.

Results

Temperature of treatment	No. of plants inoculated	No. of plants diseased	
60° C.	5 ...	2	
70° C. ...	5 ...	2	
80° C. ...	5 ...	0	
90° C. ...	5 ...	1	?

The plant showing disease in the 90° C. lot did not show typical symptoms like those of the lower temperature series which became diseased, and as there is a doubt as to the disease, it was decided to neglect this figure and to conclude that the virus extract of dock mosaic was not inactivated by a temperature of 70° C. for ten minutes, but was destroyed by heating to 80° C. for the same time.

Relation of the virus to the seed of its host

A few virus diseases of plants are known to be transmitted through the seed of their host, so it was decided to check this point for dock mosaic. Seed from a plant of *Rumex obtusifolius* L. which showed severe symptoms of mosaic was accordingly saved in the autumn of 1928, being planted in a shallow pan of soil in the spring of 1929. One hundred and twenty-seven seedlings resulted from this process, not one of which appeared to be infected with mosaic.

Further observation on seedlings growing amongst a population of adult docks with the mosaic disease showed that of 150 seedlings, not one had mosaic. It is, therefore, concluded that the virus of dock mosaic is incapable of infecting the seed.

The effect of high temperatures on the appearance of mosaic symptoms

Five diseased docks were potted up in the spring of 1929 and were grown in a cool greenhouse. The mean maximum temperature was at first about 65° F., but during July it rose to the neighbourhood of 75° F. It was noticed that growth made during this latter period showed no characteristic mosaic mottling, and that the leaves were almost uniformly green. It appears that symptoms of the mosaic under question are masked by a growth temperature in the neighbourhood of 75° F.

The effect of the disease on the pH of the host-sap

Extracts from mottled and from unmottled dock leaves were filtered through fine muslin and were separately centrifuged until clear enough to be translucent when placed in small drops on a white tile. A drop of Brom Cresol Green indicator was added to each, when the healthy extract appeared quite green, whilst the diseased liquid was definitely bluer. Comparison with colour standards suggested values of *p*H 4·8 for the healthy and *p*H 5·2 for the diseased extracts.

Validity of experimental results

The experiments were all carried out against adequate controls amounting to 20 in all. Not one of these showed any disease, so that it is concluded that such low infection as, say, two plants diseased out of five inoculated, with the same liquid, is yet quite significant.

Summary

The description of dock mosaic for diagnostic purposes is given on the lines laid down by Johnson.[2]

Symptoms on *Rumex obtusifolius* L. & *R. lanceolatus* L.—No stunting of diseased plants as compared with healthy ones has been observed, and the only disturbance is a mottle of light green spots which usually occur between the anastomosing veinlets of the leaf. Flowering, fruiting, and rooting of diseased plants appear to be quite normal.

Resistance to ageing in vitro—Virus extract not inactivated after 14 days. Dried diseased leaves still infectious after 21 days.

Thermal death-point—80° C. for 10 minutes.

Resistance to chemicals—95% Alcohol does not destroy, but 2% formalin destroys in half an hour.

Resistance to dilution—Still infectious when diluted to 1 in 100 with water, but not infectious when diluted to 1 in 1000.

Filtration—Not infectious when passed through a Jenkins filter or an English Berkefeld candle.

Temperature of masking of symptoms—75° F.

Transmission by tissue-mutilation. The virus is not seed-borne.

Effect of physiology of host—Infection with the disease causes an increase of about *p*H 0·4 in the sap as compared with healthy.

The results do not give absolute proof that the cause of the chlorosis of the dock is a virus, but it is suggested that they show a similarity with virus diseases close enough for its provisional classification with these maladies.

References

[1] Grainger, J. "An Infectious Chlorosis of the Dock." Proc. of the Leeds Philosophical Society, **1**, Pt. 8, p. 360 (1928).

[2] Johnson, J. "The Classification of Plant Viruses." Wis. Univ. Agr. Exp. Sta. Bull. No. 76, pp. 1–16 (1927).

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